Measurements of magnetic fields associated with end plate potentials induced from neuromuscular junctions in frogs

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1 Introduction

Magnetic fields induced from neuromuscular junctions in frogs were measured with a high-resolution DC-SQUID magnetometer in a magnetically shielded room. After decreasing the calcium concentration of Ringer's solution, magnetic fields associated with end plate potentials were successfully measured. The peak amplitude and the duration of the end plate magnetic fields were from 2 to 6 pT and from 40 to 50 ms, respectively.

2 Methods

2.1 Preparation

The nerve-muscle preparations of adult frogs (*Rana catesbeiana*) were carefully dissected to prevent the disconnection of the nerves and muscles. During the dissection, the nerve bundles were stimulated by a hand-made Cu-Zn twister to check the disconnection of the nerve and muscle. The muscles were discarded if they did not contract after stimulation. The successfully dissected preparations were mounted in small plastic chambers filled with Ringer's solution. Sartorius and Gastrocnemius-Solues muscles were used for the preparation, and the sciatic nerve fibers were about 3 cm to 7 cm long.

2.2 Solutions

Two types of Ringer's solution were used; a normal Ringer solution (115 mM sodium, 2.0 mM potassium, 1.8 mM calcium, 120 mM chloride), and a low calcium concentration Ringer solution (109 mM sodium, 2.0 mM potassium, 0.8 mM calcium, 5.4 mM magnesium, 120 mM chloride). The normal Ringer was used only to prevent the nerves and muscles from drying up.

2.3 Magnetic field measurement

A 12-channel DC-SQUID gradiometer with a 5 mm in diameter pickup coil and a 15 mm baseline, was used for the measurements of magnetic fields in a five-layer magnetically shielded room. The field sensitivity was 100 fT/(√Hz) in the white noise frequency region. The distance between the pickup coil and the outside surface of the SQUID dewar was 5.0 mm, and the distance between the outside surface of the SQUID dewar and the surface of neuromuscular preparation was 1.0 mm.

2.4 Electrophysiological measurement

A conventional electrophysiological measurement was carried out on the nerve preparation for comparison with the magnetic field measurement. The solutions and electrical stimulation condition were the same as those used in the magnetic field measurement. A micro-glass-electrode was inserted in the neuromuscular junction for the measurements of membrane potentials with the guidance of an optical microscope. The wire connecting the electrode to an amplifier was shielded, and the signal from the amplifier was monitored and recorded by a personal computer.

2.5 Stimulation simulation

Before the magnetic and electrical experiments, the stimulation condition was simulated by a self-made computer software, which solved the Hodgkin-Huxley nerve equations [1-4]. The program was implemented in a graphical language, LabVIEW. By varying the stimulation period and strength parameters, the optimal condition was solved in real time, set to the electric stimulator, and the pulse wave was applied to the nerve bundle.

3 Results and discussion

3.1 Reduction check

The muscles were contracted simultaneously during stimulation in normal Ringer solution with a 1.8 mM concentration of calcium. The chamber was then emptied of the normal Ringer's solution and refilled with the lower calcium concentration Ringer's solution (0.8 mM). Consequently, the muscles exhibited weak contractions during stimulation.

The magnetic fields during this time period were recorded and data was taken every 3 minutes. Fig.2

shows the averaged signal after 100 stimulations. The peak amplitude of the magnetic fields was 150 pT just after changing the solution, and the amplitudes of every SQUID channel decreased thereafter.

3.2 Polarity check

Fig. 2 shows that the polarity of channels #10 and #11 are opposite to that of channels #09 and #12. Hence, the current flowed from left to right in Fig.2. This is consistent with respect to the layout shown in Fig.1.

3.3 Latency and sloping check

As the peak amplitude decreased, the latency of the signal from the stimulus point to the spiking point increased and the slope of the spike decreased. The low calcium concentration of the extracellular solution caused a reduction in the calcium current through voltage-gated calcium ion channels on the presynaptic terminals. Consequently, a reduction in the amount of released neurotransmitter, such as acetylcholine, prevented the action potential of the muscle fiber at the neuromuscular junction to be readily evoked.

3.4 EPP search

Although the muscle did not contract 30 minutes after changing the solution, end plate potentials (EPP) were detected. Averaged signals with small amplitudes appeared at several channels.
Fig.3 shows such signals. The peak amplitude of the magnetic fields was 6pT. These channels were not the all but limited to which SQUID sensor position was near to the connection region of nerve and muscle. The channels located far from the connection region did not show EPP signals. The transient time of EPP signals were within 50 ms.

3.5 EPP profile check

The profile curve of EPP was derived by B.Katz [5], as the cable equation,

$$-\lambda^2 \frac{d^2V}{dx^2} + \tau_m \frac{dV}{dt} + V = 0$$

and its solution,

$$V = \frac{q_0}{2c_m\lambda\sqrt{(\pi t/\tau_m)}} \exp(-\frac{x^2\tau_m}{4\lambda^2 t} - \frac{t}{\tau_m})$$

where q_0 is the initial charge on the membrane, c_m is the capacity of the membrane per unit length of fiber, τ_m is the time constant of the membrane and λ is the time constant of the muscle fiber.
The observed data of both magnetic and electrophysiological experiments were tested to fit the above equation by using *Mathematica*. The profile equation can be applied to both Fig. 4 (magnetic) and Fig.5 (electrophysiological data).
Thus, the magnetic fields associated with end plate potentials induced from neuromuscular junctions were successfully measured. Biomagnetic measurements of synaptic transmission processes [6] by micro SQUID systems are useful for physiological and pharmacological studies such as stimulus-excitation-contraction coupling.

Acknowledgement

The authors thank the Nippon National Instruments Company for their helpful support and useful advice in programming with LabVIEW.

References

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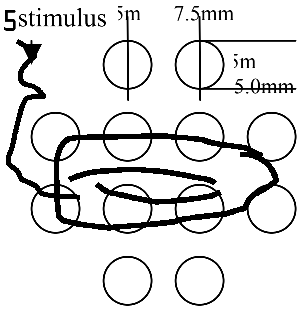
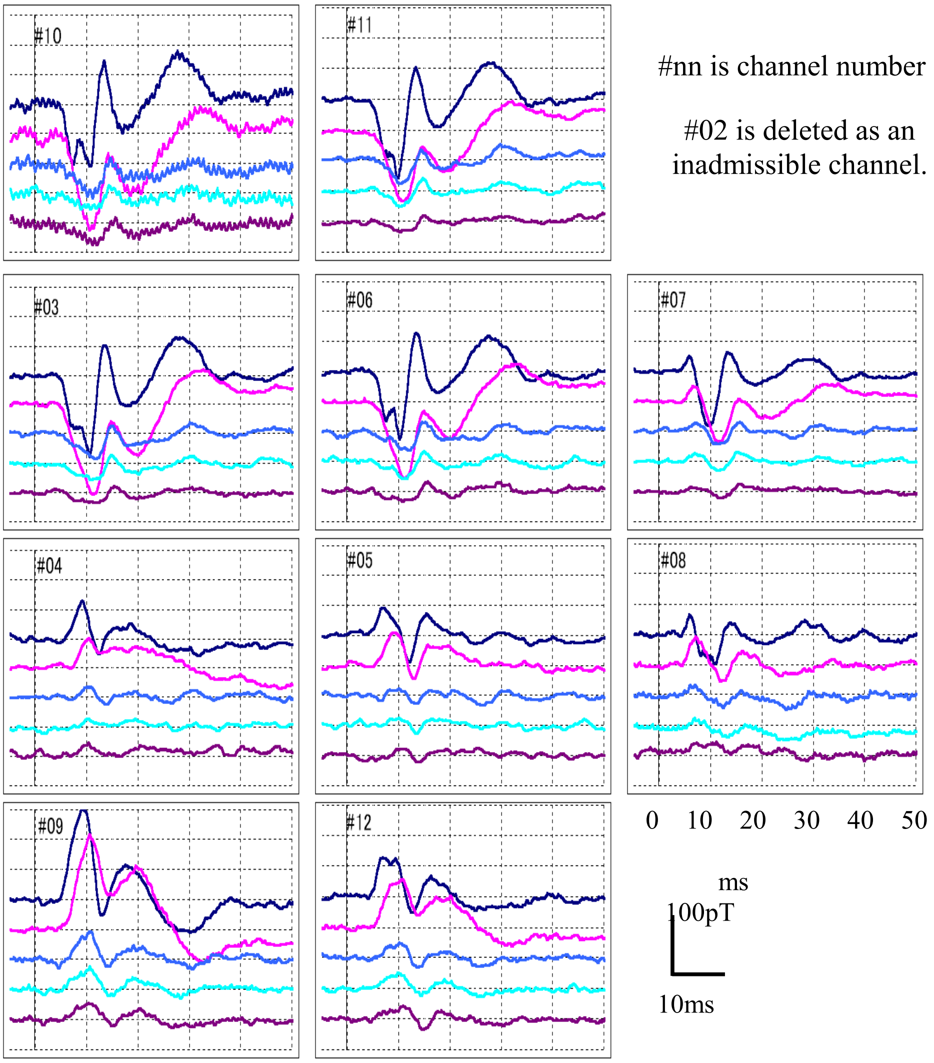
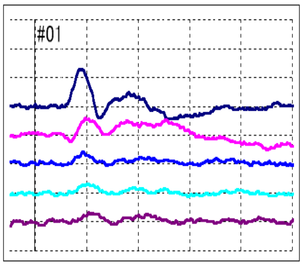


Figure 1: *Layout of SQUID sensors and nerve-muscle preparation.*





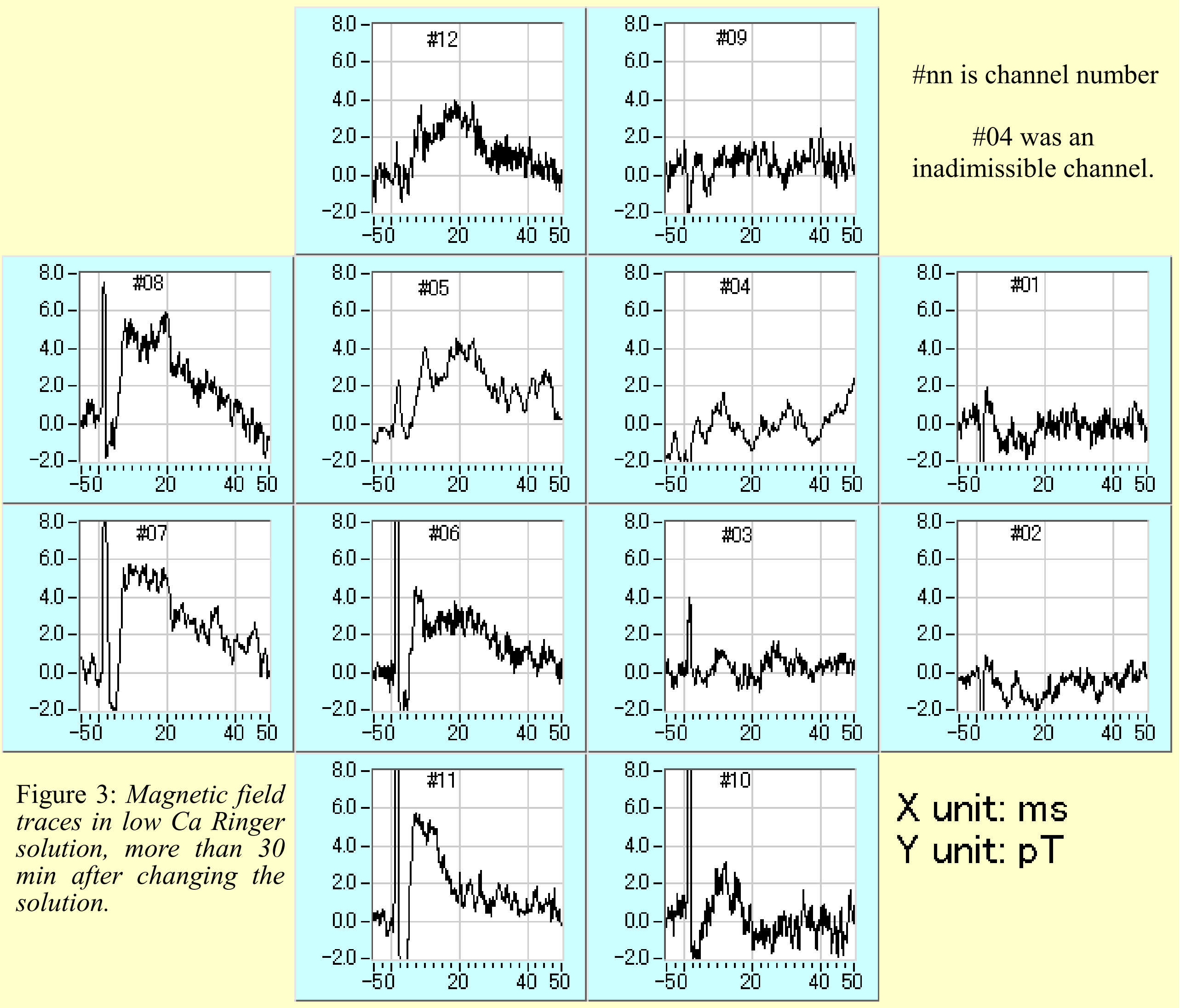
#nn is channel number

#02 is deleted as an inadmissible channel.

Figure 2: *Magnetic field traces in low Ca Ringer solution.*

3 min ---
6 min ---
9 min ---
12 min ---
15 min ---

time interval after changing the solution.



#nn is channel number

#04 was an inadimissible channel.

Figure 3: *Magnetic field traces in low Ca Ringer solution, more than 30 min after changing the solution.*

X unit: ms
Y unit: pT

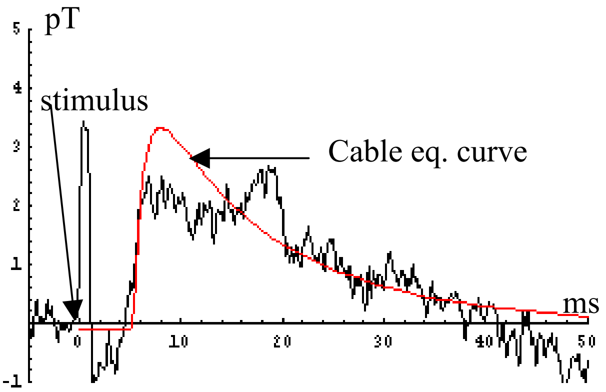


Figure 4: *Magnetic field trace in low Ca Ringer solution, more than 30 min after changing the solution.*

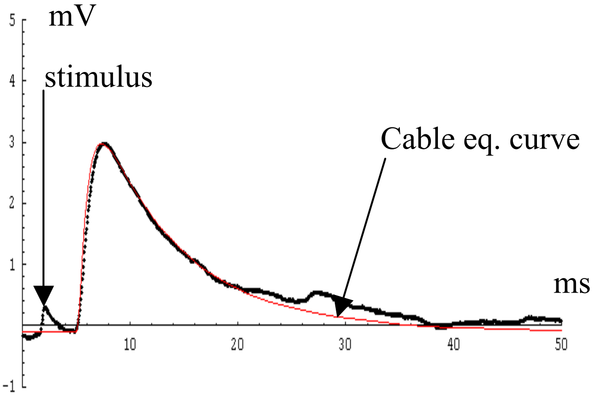


Figure 5: *End plate potential trace in low Ca Ringer solution, more than 30 min after changing the solution, measured by a micro-glass-electrode.*